region, where two genes (SYFEL and H-SPRY-3) that are subject to X inactivation have Y-linked homologs that are also silenced (Marta-Rosaria Matarazzo, International Inst. of Genetics and Biophysics, Italy). How such Y-linked silencing is achieved remains to be discovered.

The imprinted inactivation of the paternal X chromosome that occurs in marsupials and in the extra-embryonic tissues of rodents is often cited as the ancestral form of X inactivation. Nobuo Tagaki (Hokkaido University, Japan) presented data challenging this concept. In mouse XX androgensomes, which have two paternal X chromosomes, rather than inactivation of both Xs, random X inactivation of one or the other X occurred in extra-embryonic cells. This suggests that resistance of the maternal X to inactivation, rather than obligatory paternal X inactivation, underlies imprinted X inactivation. It also implies that a counting mechanism, capable of recognizing the number of X chromosomes in the cell, functions in extra-embryonic tissues, as in random X inactivation. However, the paternal X might still show some predisposition to inactivation because it is coated by Xist RNA well before the first signs of inactivation in extra-embryonic tissues. What is the nature of the X-inactivation imprint? Methylation analysis—a ‘must’ for any self-respecting imprinting story—of the Xist 5’ region revealed no obviously imprinted sites (Graham Kay, the Queensland Institute of Medical Research, Australia), but it might be that the 3’ end is where it all happens. The CpG-rich region lying 15 kb 3’ to Xist and close to the promoter of the recently described Xist antisense transcript, is one good candidate. Deletion of this region abolishes Xist sense and antisense expression in undifferentiated embryonic stem (ES) cells and also leads to non-random inactivation of the targeted allele (Phil Avner, Pasteur Institute, France). X inactivation during spermatogenesis seems to be yet another story. The transient nature of this X inactivation in male meiotic cells could be owing to the absence of methylation and histone acetylation or, more simply, to transient exclusion of RNA polymerase II from the sex vesicle (XY body). Because this X inactivation is unlikely to be regulated by Xist, as it is apparently unaffected in Xist knockout mice (John McCarron, the Southwestern Foundation for Biomedical Research, USA), the significance of the very low levels of Xist expression and possible Xist-coating of the sex vesicle observed in male meiotic cells remains unresolved, as does indeed the evolutionary significance of X inactivation during gametogenesis.

Another intriguing issue concerns which parts of the Xist gene are involved in its regulation and function. Insights are expected to come from gene targeting and evolutionary studies. One surprise is that deletion of one of the most highly conserved regions of the Xist transcript, exon 4, gives no apparent phenotype (Marie-Laure Caparros, MRC, London, UK). Another surprise is that the upstream ES-cell-specific promoter P0 region is highly repetitive and poorly conserved in vole species, unlike the P1 and P2 somatic promoters (Tatyana Nesterova, Academy of Sciences, Novosibirsk, Russia). There might even be species differences in the way Xist is regulated during development, because human XIST RNA is stable and coats autosomes in cis in undifferentiated mouse ES and embryonal carcinoma cells, unlike its mouse counterpart (Edith Heard, Pasteur Institute, France; Ikuya Yoshida, Hokkaido University, Japan). In fact, Xist still hasn’t passed the ultimate evolutionary test for mammals: is it conserved in marsupials?

Future directions in the X-inactivation field include a promising mutagenesis program reported by Laura Carrel, aimed at isolating additional X inactivation variants. Some of these mutations might be associated with the Xor locus, which has long been a paradigm for bias in X inactivation and whose map position has recently been greatly refined (Phil Avner, Pasteur Institute, France). Identification of some of the protein partners of the Xist transcript and the inactive X will also be crucial. To date, histone macroH2A is the only candidate. As it is apparently not required for maintenance of the inactive state and Jacqueline Mermoud (MRC, London, UK) showed that its colocalization with the inactive X occurs surprisingly late during in vivo differentiation, its role remains enigmatic. Integrating all this and much more suggests that understanding X inactivation, while indeed a subject fit for mammals, could require a touch of divine insight.

Further reading

Meeting Reports
Outlook

Organelle genes – do they jump or are they pushed?

R ace et al. presented a stimulating account of the likely reason for retention of genes in some organelles in September’s issue. As they made clear, this begs the question of what drives the loss of genes to the nucleus anyway. They described suggestions that the selection pressure might arise from Muller’s ratchet, exacerbated by the occurrence of DNA-damaging processes in the organelles. We would like to suggest another explanation (that might act together with Muller’s ratchet), based on our studies of plastid genomes. These genomes have a tendency to...
The Hox genes are a family of transcription factors that define specific anteroposterior identities, both in vertebrate and in invertebrate embryos, and that are characterized by a very highly conserved DNA-binding motif known as the homeodomain. In vitro, most Hox proteins recognize the same four-base-pair consensus sequence that is actually repeated many times in the genome. For greater binding specificity is achieved when Hox proteins bind as heterodimers with PBX proteins (vertebrate homologs of Drosophila extradenticle-containing transcription factor extradenticle). PBX and Hox proteins interact at a specific amino acid composition on the surface of the Hox protein. This short sequence of amino acids is necessary for PBX binding and, apart from the homeodomain itself, is the most characteristic feature of Hox genes. During the evolution of vertebrates, the ancestral cluster of Hox genes was duplicated at least twice, hence, most vertebrates have at least four independent Hox clusters, referred to as A, B, C, etc. Despite some Hox genes in each cluster having become non-functional or even entirely deleted subsequent to the duplication step, the overall genomic structure of each cluster has been conserved in evolution. In general, the descendants of each of the genes in the ancestral Hox cluster have similar expression patterns and some conserved functions. They are described as paralogs (e.g. Hoxa1, Hoxb1 and Hoxd1). Outside of the homeodomain region, the overall sequence identity between members of each paralog group is very low. As a result, the paralog identity of each gene has often been ambiguous.

Two previous studies have addressed this problem by aligning Hox genes based on their hexapeptide sequences rather than their homeodomains (which forms the usual basis for Hox gene alignment comparisons). Their findings revealed that there were, indeed, some amino acids adjacent to the hexapeptide that are conserved only within individual paralog groups. Here we have extended these studies to include all hexapeptide-containing paralog groups from a wide range of species. Interestingly, this reveals that there are several very highly conserved amino acids clustered around the hexapeptide sequence. These amino acids consistently identify Hox genes as belonging to a particular paralog group (Fig. 1).

Why are the amino acids around the hexapeptide sequence so highly conserved between paralogs but not clusters? Members of one paralog bind to a distinct DNA sequence only when bound to PBX at the hexapeptide site;

**FiguRe 1.** Conserved amino acids around the hexapeptide sequence of Hox paralog groups (1–8)

| Hox1 | TPDMVVRKNNPxP | Hox2 | PCEPWRKXXKKX | Hox3 | KIPFWMERGRRNNKXK | Hox4 | VUPWPWKRHNNNNNXY | Hox5 | PQYPWMKLRSNRKNNRXR | Hox6 | hYPWMQRMMNNSXXFXGGKKXRXR | Hox7 | RYPWMRGRGKXD | Hox8 | hFPWRMW |